

by combining a sulfation consensus sequence from one polypeptide with a glycosylation consensus sequence from a different polypeptide, by inserting such sequences into a carrier polypeptide, or by repositioning such sulfation and glycosylation consensus sequences, relative to one another, within the same polypeptide. In general, therefore, applicants' claimed invention features purified nucleic acids encoding artificial P-selectin ligand polypeptides that contain amino acid consensus sequences for attachment of sialyl Le<sup>x</sup> and sulfate groups, wherein at least one of the consensus sequences is located at a non-naturally occurring amino acid position. The invention also features vectors and cells containing the claimed nucleic acids.

#### Summary of the Office Action

Claims 10 and 12-14 stand rejected under 35 U.S.C. §§ 102, 103, and 112, first and second paragraphs. In addition, amendments to the specification and a new title are required.

#### Support for the Amendments

Claims 10 and 12 have been amended. Support for the amendment to claim 10 may be found, e.g., at page 24, lines 1-23, and at Fig. 14. Support for the amendment to claim 12 may be found, e.g., at page 24, lines 17-27 and at page 28, line 5, through page 29, line 14.

For the record, applicants note that they do not agree with the current rejections and reserve the right to pursue the canceled subject matter in this or related applications.

#### Drawings

Applicants will provide formal drawings when otherwise-allowable subject matter has been indicated.

#### Rejections under 35 U.S.C. § 112, first paragraph

Claims 10 and 12-14 stand rejected under 35 U.S.C. § 112, first paragraph. The Office Action states that there is insufficient guidance to assist one skilled in the art to select and use appropriate "organic molecules" encompassed by the claimed invention and that, therefore, claims reciting nucleic acids encoding such organic molecules are not enabled.

In response, applicants have amended claim 10 and dependent claims 12-14, and the rejection may now be withdrawn. In particular, claim 10 now specifies a purified nucleic acid encoding a polypeptide that is an artificial P-selectin ligand. The encoded polypeptide contains amino acid consensus sequences for attachment of sialyl Le<sup>x</sup> and sulfate moieties, and at least one of the consensus sequences is located at an amino acid position at which it does not naturally occur.

These claims are clearly enabled by the present specification. Applicants have

provided examples of nucleic acids encoding artificial P-selectin ligand polypeptides of the type now recited by the claims. For instance, one exemplary nucleic acid (described at page 24, lines 1-16) encodes the Factor VIII sulfation consensus sequence fused upstream from a nucleic acid encoding a CD43 fragment consisting of Ile135 through the carboxy terminus of CD43 (encompassing the membrane proximal, transmembrane, and intracellular domains of CD43). This nucleic acid construct encodes an artificial polypeptide, in which the CD43 glycosylation sequences of the proximal domain are fused to a sulfation consensus sequence located at an amino acid position within the CD43 polypeptide at which this sulfation sequence does not naturally occur. This polypeptide functions as an artificial P-selectin ligand.

Additional nucleic acids encoding artificial P-selectin ligands may be similarly generated using any polypeptide coding sequence as a scaffold for introduction of sulfation and sialyl Le<sup>x</sup> consensus sequences. One of skill in the art would know that it is necessary to position the sulfation and sialyl Le<sup>x</sup> consensus sequences such that any added sulfate and sialyl Le<sup>x</sup> moieties are exposed on the outer surface of the polypeptide to be used as a scaffold. The appropriate positions for introduction of consensus sequences are easily determined using well-known algorithms, and polypeptides encoded by the claimed nucleic acids are easily tested for binding to P-selectin by methods described in the specification and known in the art. Accordingly, claim 10, as amended, and dependent claims 12-14, are enabled under 35 U.S.C. § 112, first paragraph, and this rejection may

be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 10 and 12-14 also stand rejected under 35 U.S.C. § 112, second paragraph. Specifically, the Office Action states that the claims are indefinite because: (a) claim 10 should be written in an independent format; (b) it is not clear how the recitation of "non-naturally occurring sites" and "covalently bonded determinants" in claim 1 (from which claim 10 depends) relates to nucleic acids because sialyl Le<sup>x</sup> and sulfate determinants are not required by the claimed invention drawn to nucleic acids; and (c) it is not clear whether the nucleic acids of claim 12 encompass entire antibody molecules or, instead, antibody domains (e.g., for use in immunoglobulin fusion proteins).

In response to the Examiner's first two points, applicants have amended claim 10 such that it is now an independent claim that no longer recites "non-naturally occurring sites" and "covalently bonded determinants." With respect to the statement in the Office Action that sialyl Le<sup>x</sup> and sulfated determinants are not required by the claimed invention drawn to nucleic acids, the claims now recite nucleic acids encoding amino acid sequences for attachment of sialyl Le<sup>x</sup> and sulfate moieties. The rejection, as applied to claim 10, may be withdrawn.

With respect to the statement that claim 12 is indefinite because it is unclear whether the claimed nucleic acids encode entire antibody molecules or antibody domains,

applicants have amended claim 12 to specify that this claim encompasses both full-length antibodies and antibody fusion molecules. Moreover, the term "fusion molecules" is meant to include polypeptides containing either whole antibodies or antibody domains (see page 29, lines 6-14 of the specification). Support for the term "fusion molecules," as described above, is found at page 24, lines 17 through 27, and page 28, line 5, through page 29, line 14.

The passage on page 24, lines 17-27, clearly referring to entire antibody molecules and fusion proteins encompassing entire antibody molecules, states that antibodies bearing both sialyl Le<sup>x</sup> and sulfate groups may be created by introducing sulfation sites into an existing antibody molecule in the vicinity of an introduced or existing sialyl Le<sup>x</sup> addition site, for example, by site-directed mutagenesis. Alternatively, any P-selectin ligand sequence may be appended to a naturally-occurring antibody sequence. The passage on page 28, line 21, through page 29, line 5 addresses fusion molecules containing antibody domains and provides, as examples, antibody fusion proteins containing  $\alpha_1$ -acid glycoprotein fused to antibody domains composed of the hinge-CH2-CH3 or CH2-CH3 regions. The passage on page 29, lines 6-14, states that fusion proteins containing whole antibodies or antibody domains can be modified by adding sulfation and sialyl Le<sup>x</sup> consensus sequences in order to render the antibody fusion proteins capable of blocking P-selectin-mediated interactions. Such sequences are introduced using standard techniques of recombinant DNA technology.

In sum, the specification teaches whole antibody molecules as well as fusion molecules containing either whole antibodies or antibody domains. All of these molecules may be modified by the addition of sulfate and sialyl Le<sup>x</sup> consensus sequences for use as artificial P-selectin ligands. Applicants' amendment to claim 12 reflects this teaching in the specification.

In view of the above discussion and the amendments to claims 10 and 12, the § 112, second paragraph rejection may be withdrawn.

#### Rejections under 35 U.S.C. § 102

Claims 10 and 12-14 also stand rejected, under 35 U.S.C. § 102(e), as being anticipated by Seed et al. (U.S. Patent No. 5,723,583; "Seed"), Newman et al. (U.S. Patent No. 5,681,722; "Newman"), or Lasky et al. (U.S. Patent No. 5,304,640; "Lasky"); under 35 U.S.C. § 102(a), as being anticipated by Lasky; and, under 35 U.S.C. § 102(b), as being anticipated by Larsen et al. (WO 94/10309; "Larsen"), Sasaki et al. (*J. Biol. Chem.* 269:14730, 1994; "Sasaki"), or Meier et al. (*Biochem. J.* 294:25-30, 1993; "Meier"). These rejections are respectfully traversed.

The claims, as amended, now require that the claimed nucleic acids encode polypeptides containing consensus sequences for attachment of sialyl Le<sup>x</sup> and sulfate moieties, at least one of which is at an amino acid position at which it does not naturally occur. Because none of the cited references includes each and every one of these claim

limitations, they cannot anticipate applicants' presently claimed invention.

In particular, although the nucleic acids of the first cited reference, Seed, encode polypeptides having sialyl Le<sup>x</sup> addition sites, no mention is made in this reference of sulfation addition sites. Furthermore, such sites are not expected or necessary within the Seed antibody molecules, as these polypeptides are engineered for binding to ELAM-1 (E-selectin), and E-selectin ligands do not require sulfation for binding activity. Clearly, the Seed nucleic acids are not the same as the nucleic acids recited in the amended claims. Therefore, claim 10 and dependent claims 12-14, as amended, are not anticipated by Seed, and this basis for the rejection may be withdrawn.

The second cited reference, by Newman, teaches chimeric antibodies that include variable regions from Old World monkey antibodies and constant regions from human antibodies, nucleic acids encoding such chimeric antibodies, and vectors and host cells containing the nucleic acids. Newman, like Seed, makes no mention of sulfation consensus sequences. Therefore, claims 10 and 12-14, as amended, cannot be anticipated by Newman, and this basis for rejection may also be withdrawn.

The third cited reference, by Lasky, similarly fails to anticipate claims 10 or 12-14. Lasky teaches nucleic acids encoding immunoglobulin fusion proteins containing GLYCAM-1, an L-selectin ligand, as well as vectors and host cells containing such nucleic acids. Lasky does not teach nucleic acids that encode selectin ligands having consensus sequences for both sialyl Le<sup>x</sup> and sulfate addition, wherein at least one of the

consensus sequences is located at an amino acid position at which it does not naturally occur. As amended, therefore, the limitations of claims 10 and 12-14 are not met by the Lasky reference, and this basis for the rejection may also be withdrawn.

Claims 10 and 12-14 stand further rejected as anticipated by Larsen. Larsen teaches P-selectin ligands fused to carrier molecules, such as immunoglobulins, but these fusions contain only naturally occurring consensus sites. Because the amended claims require nucleic acids encoding polypeptides that have at least one consensus sequence at a non-naturally occurring site on the molecule, Larsen does not anticipate the invention, and this basis for the rejection may also be withdrawn.

The fifth cited reference, by Sasaki, teaches the cloning of a novel isoform of fucosyltransferase, Fuc-TVII, and shows that expression of Fuc-TVII in human Burkitt lymphoma cells results in the increased binding of the cells to E-selectin. Sasaki also teaches nucleic acids encoding Fuc-TVII. Sasaki, however, does not teach purified nucleic acids that encode modified selectin ligands, nor does Sasaki teach vectors or host cells containing such purified nucleic acids. Claims 10 and 12-14, as amended, are therefore not anticipated by Sasaki, and this aspect of the rejection may also be withdrawn.

Finally, claims 10 and 12-14 are rejected as anticipated by Meier. Meier teaches a fusion protein that contains the first 92 amino acids of LFA3 fused to the human IgG1 hinge and heavy chain constant domain 2 and 3 regions. The recombinant fusion protein,



which behaves as an E-selectin ligand, contains six potential N-linked glycosylation sites: six in the LFA3 portion and two in the IgG portion. The Office Action states that, although the reference does not provide nucleic acids per se, the reference directs the ordinary artisan to nucleic acids encoding the modified fusion proteins.

As amended, the present claims are not anticipated by Meier. Meier does not disclose polypeptides having consensus sequences for sialyl Le<sup>x</sup> and sulfate addition, wherein at least one of the consensus sequences is located at an amino acid position at which it does not naturally occur. Indeed, Meier does not disclose a sulfation sequence at all, and, in fact, sulfate groups are not involved in recognition of ligands by E-selectin. The § 102 rejection based on Meier may be withdrawn.

#### Rejections under 35 U.S.C. § 103

Claims 10 and 12-14 stand rejected, under 35 U.S.C. § 103, as obvious over Meier or, in the alternative, as obvious over Lasky, Larsen, or Seed, in view of Sasaki, Meier, Norgard et al. (*Proc. Nat. Acad. Sci. USA* 90:1068-1072, 1993; "Norgard"), or Natsuka et al. (*J. Biol. Chem.* 269:16789-16794, 1994; "Natsuka").

With respect to the rejection over Meier, the Office Action states that it would have been obvious to obtain the claimed nucleic acids, given the teachings of this reference. As discussed above, Meier does not disclose or in any way mention polypeptides having both sulfation and sialyl Le<sup>x</sup> consensus sequences. In fact, Meier

does not in any way discuss sulfation or sulfation consensus sites, or suggest that they might be combined with sialyl Le<sup>x</sup> sites for any purpose, much less applicants' purpose of creating an artificial P-selectin ligand. In view of this deficiency in the Meier reference, it cannot render obvious the presently claimed invention.

With respect to the obviousness rejection over Lasky, Larsen, or Seed, in view of Sasaki, Meier, Norgard, or Natsuka, the Examiner is first directed to the discussion of Lasky, Larsen, Seed, Sasaki, and Meier presented above. As pointed out by applicants, none of these references, alone or in combination, teaches or suggests a nucleic acid encoding a polypeptide having non-naturally occurring consensus sequences for sialyl Le<sup>x</sup> and sulfate addition, and neither the Norgard nor the Natsuka references cures this deficiency.

In particular, Norgard discloses selective oxidation of sialic acids on the lymph node HEV ligand as a means of enhancing the interaction between L-selectin and the HEV ligand in the lymph node. And Natsuka discloses a novel fucosyltransferase (FucTVII) which, when expressed in COS-7 cells, converts the cells to sialyl Le<sup>x</sup> positivity. Neither Norgard nor Natsuka, however, teaches or suggests a nucleic acid encoding a polypeptide having non-naturally occurring consensus sequences for sialyl Le<sup>x</sup> or sulfate addition.

Accordingly, claims 10 and 12-14 cannot be rendered obvious by a combination of these cited references, and the § 103 rejection may be withdrawn.

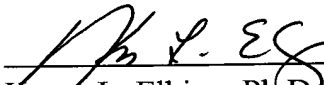
Conclusion

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested.

Enclosed is a petition to extend the period for replying for three months, to and including October 15, 1998. If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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